Risk assessment for theoretical gene drive

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What makes a good risk assessment?

Science quality criteria

- transparent and repeatable: would other investigators be able to duplicate your result?
- falsifiable: makes predictions that are measurable and (at least theoretically) falsifiable?

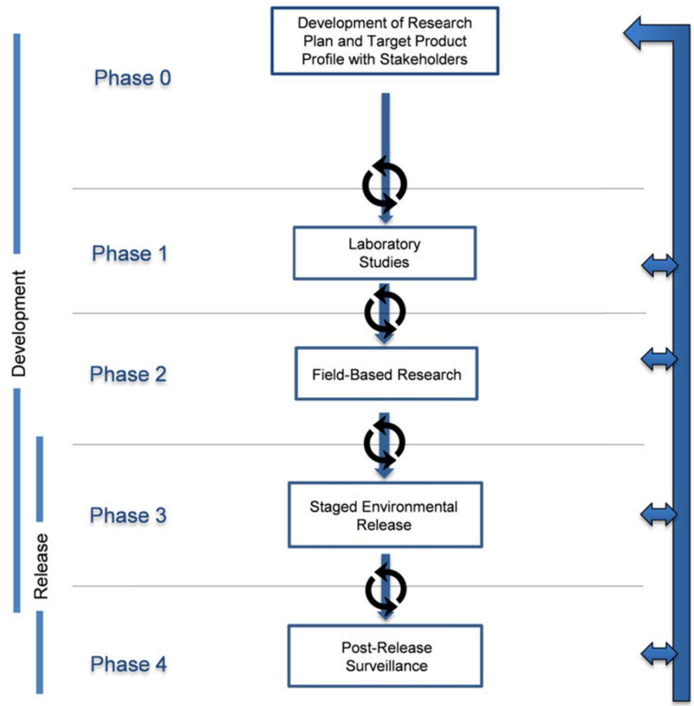
Decision quality criteria

- precision: does it provide estimates with tight confidence intervals?
- accurate: are its estimates correct?

Honest risk assessments are (Burgman, 2005):

- faithful to the assumptions about the kinds of uncertainty embedded in the assessment;
- carry these uncertainties through the analysis; and,
- represent and communicate them reliably and transparently.

NASEM raises the bar for gene drive



Phase 0: Research Preparation
- Develop a Target Product Profile
- Identify and plan for regulatory requirements
- Use models to inform standards, thresholds of acceptance, and study design
- Establish site-selection criteria (if research includes phase 2-4 trials)
- Identify risk assessment needs
- Identify appropriate confinement and containment strategies

Phase 1: Laboratory-Based Research
- Acquire required laboratory regulatory approvals
- Develop containment strategies
- Develop mitigation strategies
- Detect and measure off-target effects
- Optimize design of guide RNAs (when using CRISPR/Cas9-based gene drives)
- Utilize an optimized endonuclease with high cutting efficiency and accuracy
- Optimize for the use of homology-directed repair versus non-homologous end joining in order to maximize precision of editing
- Evaluate effects on organismal fitness in the presence of the gene drive
- Evaluate gene drive stability over multiple generations
- Mark gene drive organisms
- Use quantitative and computational methods
- Set baseline population-level effects

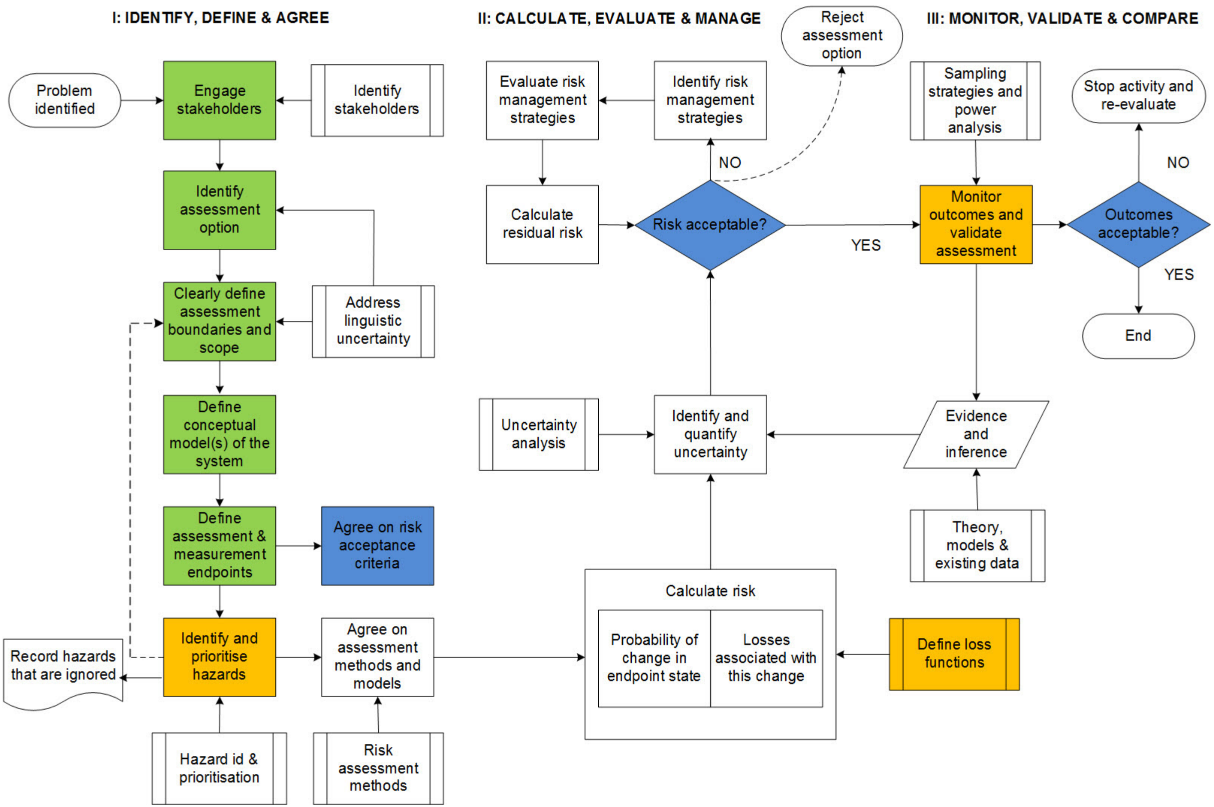
Phase 2: Field-Based Research
- Acquire site-specific regulatory approvals
- Validate efficacy
- Validate population-level effects
- Estimate impact on selected non-targets

Phase 3: Staged Environmental Release
- Acquire site-specific regulatory approvals
- Conduct monitoring and surveillance for efficacy
- Conduct monitoring and surveillance for harms

Phase 4: Post-Release Surveillance
- Acquire regulatory approvals
- Conduct monitoring and surveillance
- Measure impact

Source: NASEM (2016)

Idealised risk assessment process



Assessment endpoints: What do stakeholders care about?

Issue	Concern	Endpoint
Human health	Could other diseases emerge from the decrease of mosquitoes	Persistence of transgenic mosquitoes
Human health	Will vector competency of transgenic mosquitoes be altered	Probability of enhanced transmission capacity
Persistence	How can you monitor persistence of flying animals	Persistence of transgenic mosquitoes
Persistence	Sterility not complete and males are able to reproduce and persist	Persistence of transgenic mosquitoes
Persistence	Transgenic mosquitoes have different resistance to insecticide	Probability of enhanced insecticide resistance
Environment	Sterility will affect all mosquito species	Persistence of transgenic mosquitoes
Environment	Sterile males will effect non-target species	Persistence of transgenic mosquitoes

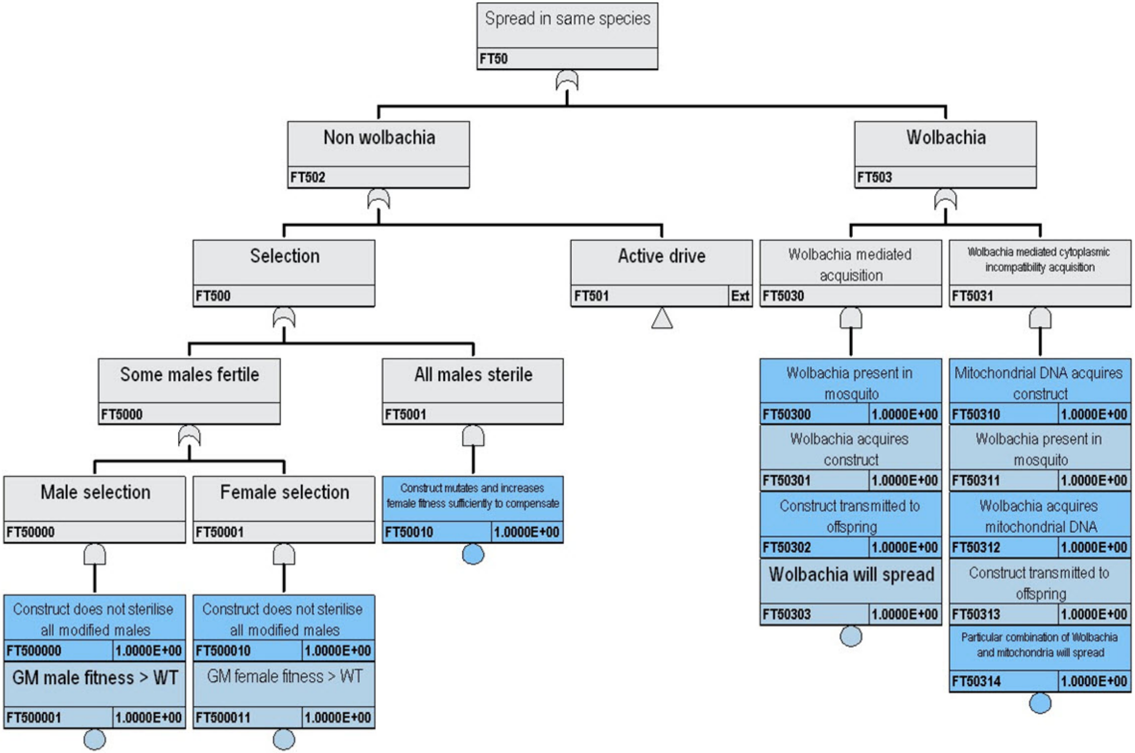
Hazard analysis for synthetic gene drive

Scale	Failure mode	Possible effect	Reference
Individual	Mutated gRNA causes Cas9 cleavage of non-target sequence	New phenotype; deleterious effect on host, such as increased virulence	Sander and Joung (2014)
	Cas9 fails to edit or target all alleles	Mosaicism within organisms; reduced drive or gain of function polymorphism	Araki et al. (2014)
	Mutations occur during repair of multiple cleavage sites	Multiple alleles leading to mosaicism in subsequent generations; reduced drive or gain of function polymorphism	Sander and Joung (2014)
Population	Assortative mating between new phenotypes	Drive is reduced or competitive advantage accrues to more virulent phenotype	Scott et al. (2002)
	Intraspecific (admixture) and interspecific hybridization	Gene drive is acquired by, and spreads within, non-target population	David et al. (2013)
	Unpredicted phenotypes arise due to gene by environment interactions	Drive failure or failure to produce refractory organisms in the wild	Tabachinck (2003)
	CRISPR/Cas9 influences the innate immune response of the GDMO	GDMO transmission of other pathogens is enhanced	Scott et al. (2002)
Community	Suppression drive creates open ecological niche	Niche filled by a more detrimental species	David et al. (2013)
	Incomplete suppression via (for e.g.) increase in drive resistant individuals	Loss of herd immunity and disease resurgence	Webb (2011)
	Horizontal transfer of gene drive to distant species	Gene drive is acquired by, and spreads within, non-target population	Wijayawardena et al. (2013)

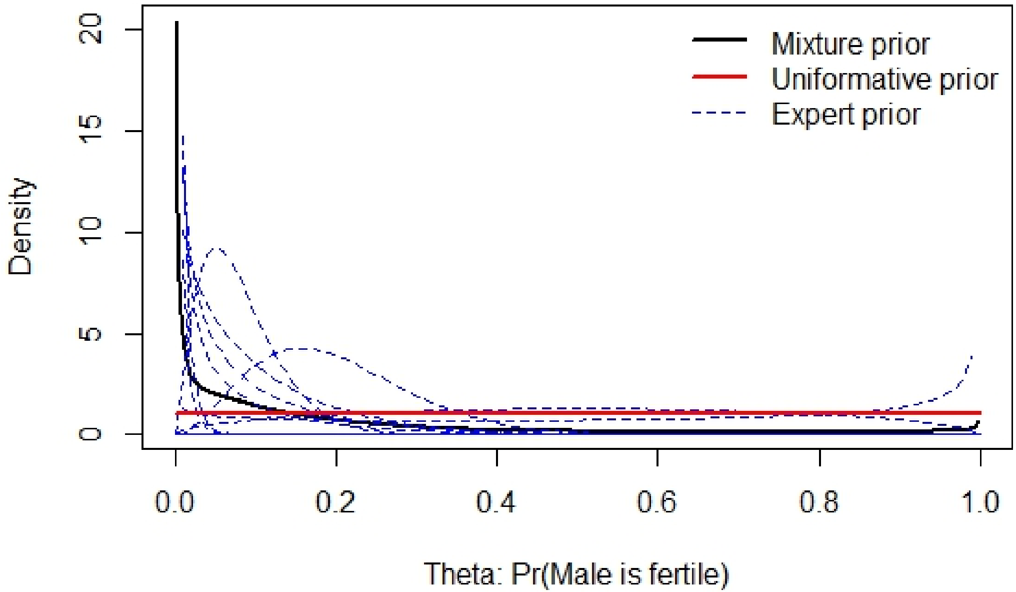
Comprehensive hazard analysis methods

Method	Application to Living Modified Organisms	Reference
Hazard and Operability Study (HAZOP)	Modified version of HAZOP, termed GENHAZ recommended as hazard identification tool for LMOs by a United Kingdom Royal Commission on Environmental Pollution	Watts (1989)
Fault Tree Analysis (FTA)	Applied as heuristic hazard identification tool for the release of genetically modified carp, and as a risk quantification tool to male sterile mosquitoes modified with I-PpoI construct	Hayes et al. (2015, 2013)
Event Tree Analysis (ETA)	Simple examples applied to biological systems - no known examples of application to LMOs	Ericson (2005)
Hierarchical Holographic Modelling (HHM)	Applied to identify hazards associated with breaches of HT Canola license conditions and male sterile mosquitoes modified with I-PpoI construct	Hayes et al. (2004, 2015)
Failure Modes and Effects Analysis (FMEA)	Modified versions applied to biological systems (hull-fouling introductions) - no known examples of application to LMOs	Hayes (2002)
Qualitative mathematical modelling (QMM)	Numerous examples of application within ecological systems, together with demonstration of application to the release of genetically modified carp in Australia	Dambacher et al. (2003); Hayes et al. (2013)

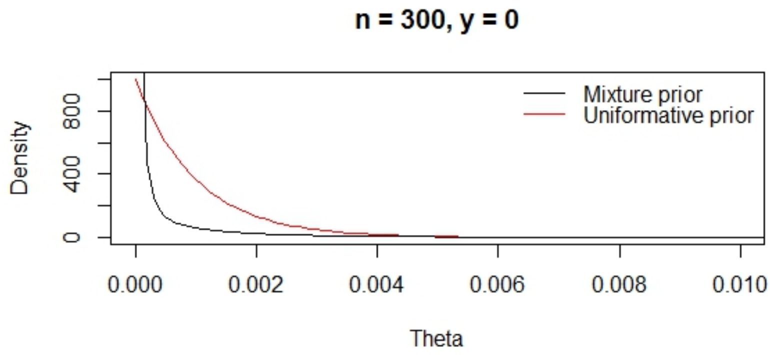
Fault tree analysis for spread of construct

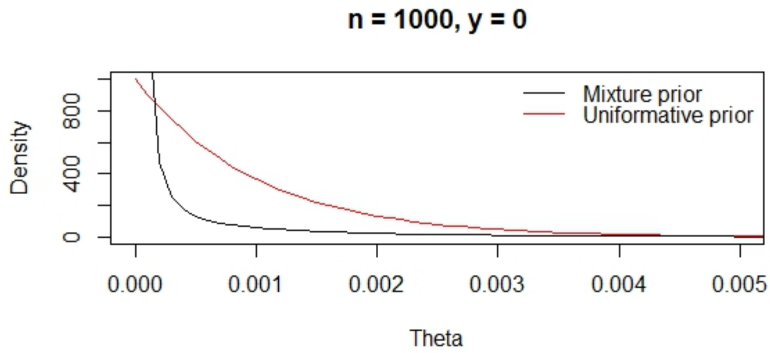


Expert elicitation: Probability male not sterile

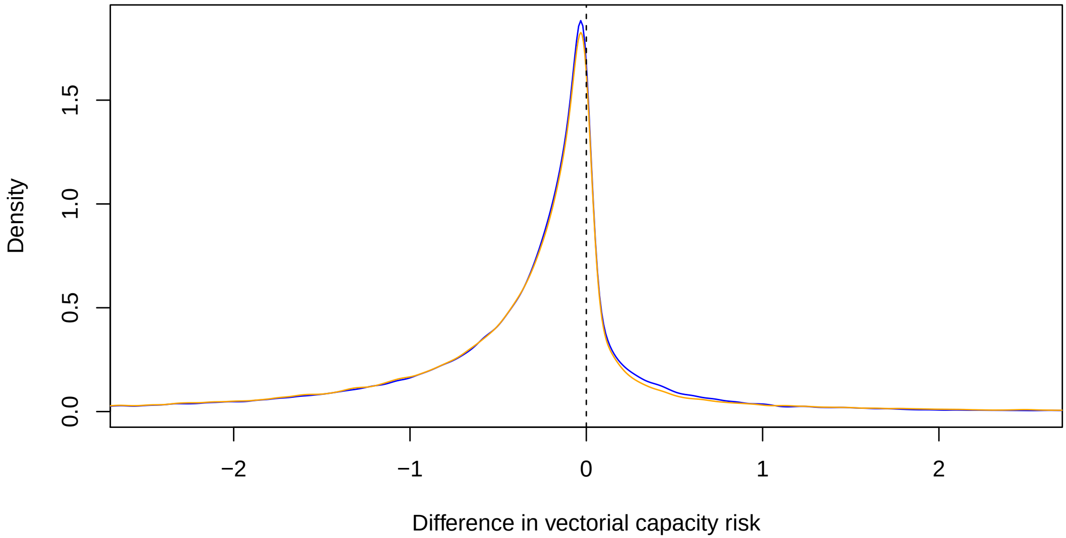


Bayesian learning with evidence of absence



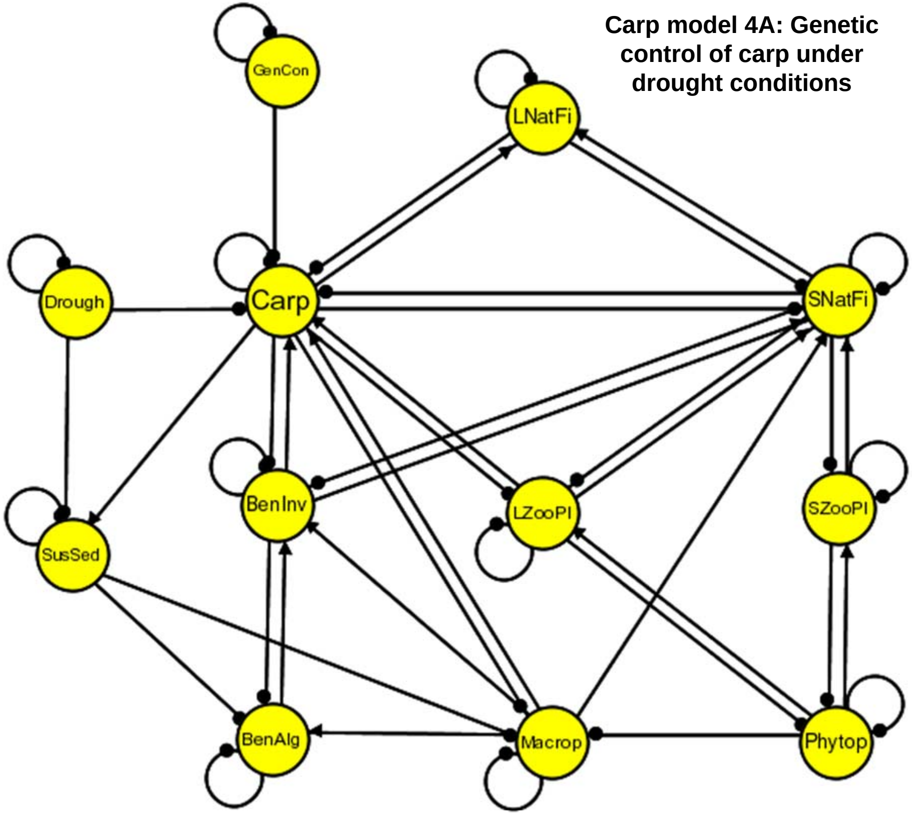


Malaria vectorial capacity WT v G3 v Transgenic

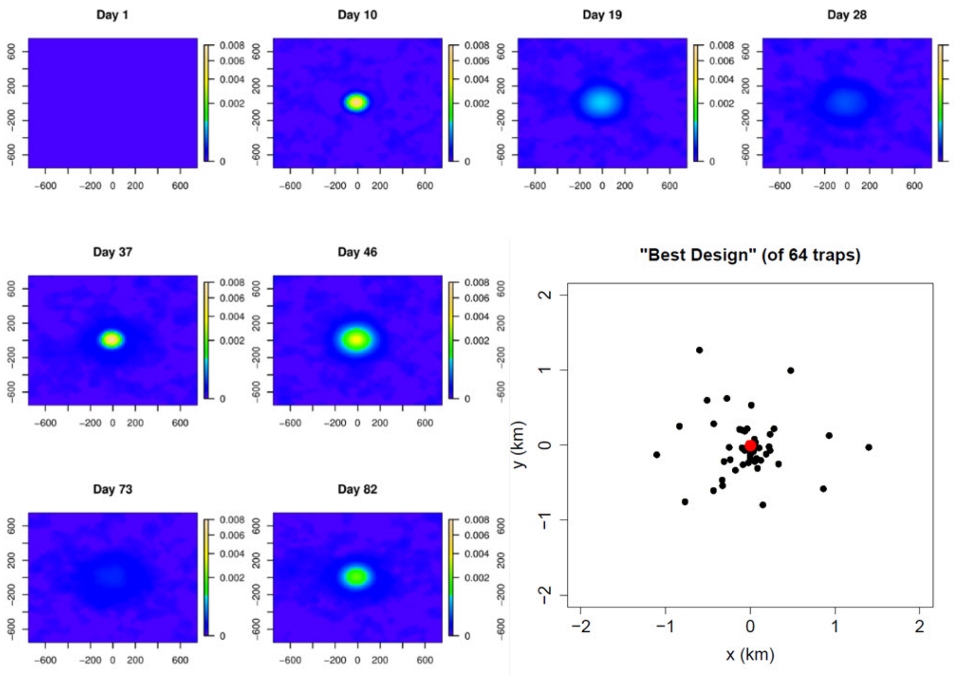


QMM: Ecosystem effects of species removal

Carp model 4A: Genetic control of carp under drought conditions



Model assisted monitoring design



Three take home messages

NASEM and gene-drive RA

- recommends a departure from qualitative status-quo

Stakeholder participation

- should help avoid involuntary risk reaction

Scientific risk assessment:

- is a verb not a noun

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Thank You

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